

## Ultrastructure of the Female Reproductive System of the Lesion Nematode, *Pratylenchus penetrans* (Nemata: Pratylenchidae)

BURTON Y. ENDO<sup>1</sup>, ULRICH ZUNKE<sup>2</sup>, AND WILLIAM P. WERGIN<sup>1,3</sup>

<sup>1</sup> U.S. Department of Agriculture, Agricultural Research Service, Plant Sciences Institute, Nematology Laboratory, Beltsville, Maryland 20705-2350, U.S.A., and

<sup>2</sup> Universität Hamburg, Institut für Angewandte Botanik, Marseiller Str. 7, 20355, Hamburg, Germany

**ABSTRACT:** Transmission electron microscopy of the reproductive system of adult females of *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven revealed details of oocyte development and the transformation of oocytes into eggs. Oogonial cell divisions were not observed; however, oogonial development into oocytes was distinctive in that most of the nuclei of ovarian cells were in the pachytene stage (i.e., prophase I of meiosis). In the midsection of the ovary, the oocytes increase in number, enlarge, and accumulate in a single row. Next, the oocytes enter a muscular oviduct and begin to accumulate lipid bodies and protein granules. The plasma membrane of the oviduct becomes plicated and forms cisternae; centralized membrane junctions establish openings for oocytes to enter the spermatheca. Spermatozoa traverse the lumen of the uterus and accumulate in the spermatheca. Each oocyte then passes through the spermatheca proximally and then traverses between columnar cells. The posteriad regions of the columnar cells attach to other uterine cells to form the central lumen of the uterus that extends beyond the vaginal opening and into the postvulvar uterine branch of the reproductive system. The fertilized egg is deposited to the exterior after passing between cuticle-lined vaginal and vulval walls supported by anteriad and posteriad muscle bands, which have ventrosublateral insertions on the body wall.

**KEY WORDS:** transmission electron microscopy, lesion nematode, female reproductive system, *Pratylenchus penetrans*, Nemata, Pratylenchidae.

The lesion nematodes, *Pratylenchus* spp., are among the most destructive plant pathogenic nematodes world-wide (Mai et al., 1977; Dropkin, 1989; Zunke, 1990a). Dropkin (1989) reviewed the disease symptoms and pathogenesis of *Pratylenchus* species, which occur as single parasites or in combination with other pathogens. The ectoparasitic and endoparasitic feeding behavior of *Pratylenchus penetrans* (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941, has been studied using video-enhanced contrast light microscopy (Zunke and Institut für den Wissenschaftlichen Film, 1988; Zunke, 1990b) and transmission electron microscopy (TEM) (Townshend et al., 1989). Light microscopic studies also have described embryogenesis and postembryogenesis, including the molting process and the development of the reproductive system, in several species of *Pratylenchus* (Roman and Hirschmann, 1969a, b). In a related study of *Ditylenchus trifurmis*, Hirschmann (1962) illustrated the development of male and female reproductive systems during postembryogenesis, beginning with the genital primordium. Recently, we used TEM to describe the

general anatomy of *P. penetrans* (Endo et al., 1997) and the development of the testis, including the production and morphology of spermatozoa (Endo et al., 1998). These observations complement extensive studies on spermatogenesis and sperm ultrastructure of various species of cyst nematodes (Shepherd et al., 1973; Cares and Baldwin, 1994a, b, 1995). To extend these studies, TEM was used to describe the ultrastructure of the female reproductive system of *P. penetrans*, with emphasis on oocyte development in the ovary and the morphology of the oviduct, spermatheca, columnar cells, and central uterus. The studies of development of the eggs include evaluation of egg shell depositions in the uterus and the vaginal and vulval muscle morphology as they relate to egg laying.

### Materials and Methods

Infective and parasitic stages of *P. penetrans* were obtained from root cultures of corn (*Zea mays* Linnaeus 'Ichief') grown in Gamborg's B-5 medium without cytokinins or auxins (Gamborg et al., 1976). Adults and juveniles were collected from infected root segments that were incubated in water. The samples were prepared for electron microscopy as previously described (Endo and Wergin, 1973; Wergin and Endo, 1976). Briefly, nematodes, which were embedded in 2% water agar or in infected roots, were chemically

<sup>3</sup> Corresponding author.

fixed in buffered 3% glutaraldehyde (0.05 M phosphate buffer, pH 6.8) at 22°C for 1.5 hr, washed for 1 hr in 6 changes of buffer, postfixed in buffered 2% osmium tetroxide for 2 hr, dehydrated in an acetone series, and infiltrated with a low-viscosity embedding medium (Spurr, 1969). Silver-gray sections were cut on an ultramicrotome with a diamond knife and mounted on uncoated 75 × 300 mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed in a Philips 400T<sup>®</sup> electron microscope operating at 60 kV with a 30- $\mu$ m objective aperture.

## Results

The female reproductive system of *P. penetrans* has amphidelphic development during early stages of postembryogenesis. However, later in the adult development, the posterior region of the ovary becomes reduced to a postvulvar uterine branch (Fig. 1). This change results in a telogonic gonad having a prodelphic orientation and a short postvulvar uterine branch that consists of epithelial cells. The cells in the anterior terminus of the ovary have spheroid nuclei, numerous polyribosomes, and high concentrations of rough endoplasmic reticulum (RER), mitochondria, Golgi, and electron-dense granules (Fig. 2 on Foldout 1). These germinal cells are completely ensheathed by spindle-shaped epithelial cells (Figs. 2, 3, 5, 6 on Foldout 2) that lie adjacent to and between the ovarian cells. In longitudinal view, the anterior gonad occupies about half the diameter of the body cavity (Figs. 1, 6, 7). Nuclear divisions of oogonia were not observed in the specimens studied. However, as the ovarian cells increase in number and size, the germ cells contribute to a double row of overlapping oogonia (Figs. 3–5). Posteriorly, oocytes occur in a single row in the ovary and attain a slightly larger size than the germinal cells in the anterior region (Figs. 6–8 on Foldout 3). The cellular organelles of the oocytes found in the midregion and proximal sites of the ovary are similar to those present in the oogonia (Figs. 2–5). The well-defined nuclei of oocytes in the midregion of the ovary contain fragments of synaptonemal complexes, indicating that the oocytes are at the pachytene stage of prophase I (Figs. 4, 5). The synaptonemal complex is a tripartite structure consisting of a central scalariform element and a pair of lateral elements. This complex is surrounded by condensed chromatin (Fig. 5). The nucleoli are prominent, large, and electron-dense (Figs. 3, 5–7). Nuclei occupy a major part of the enlarged volume of oocytes in the proximal region of the ovary (Fig. 7). In ac-

tively reproducing females, oocytes near the anterior entrance of the oviduct or within the oviduct channel have an accumulation of electron-translucent lipid droplets (Fig. 10).

## Oviduct

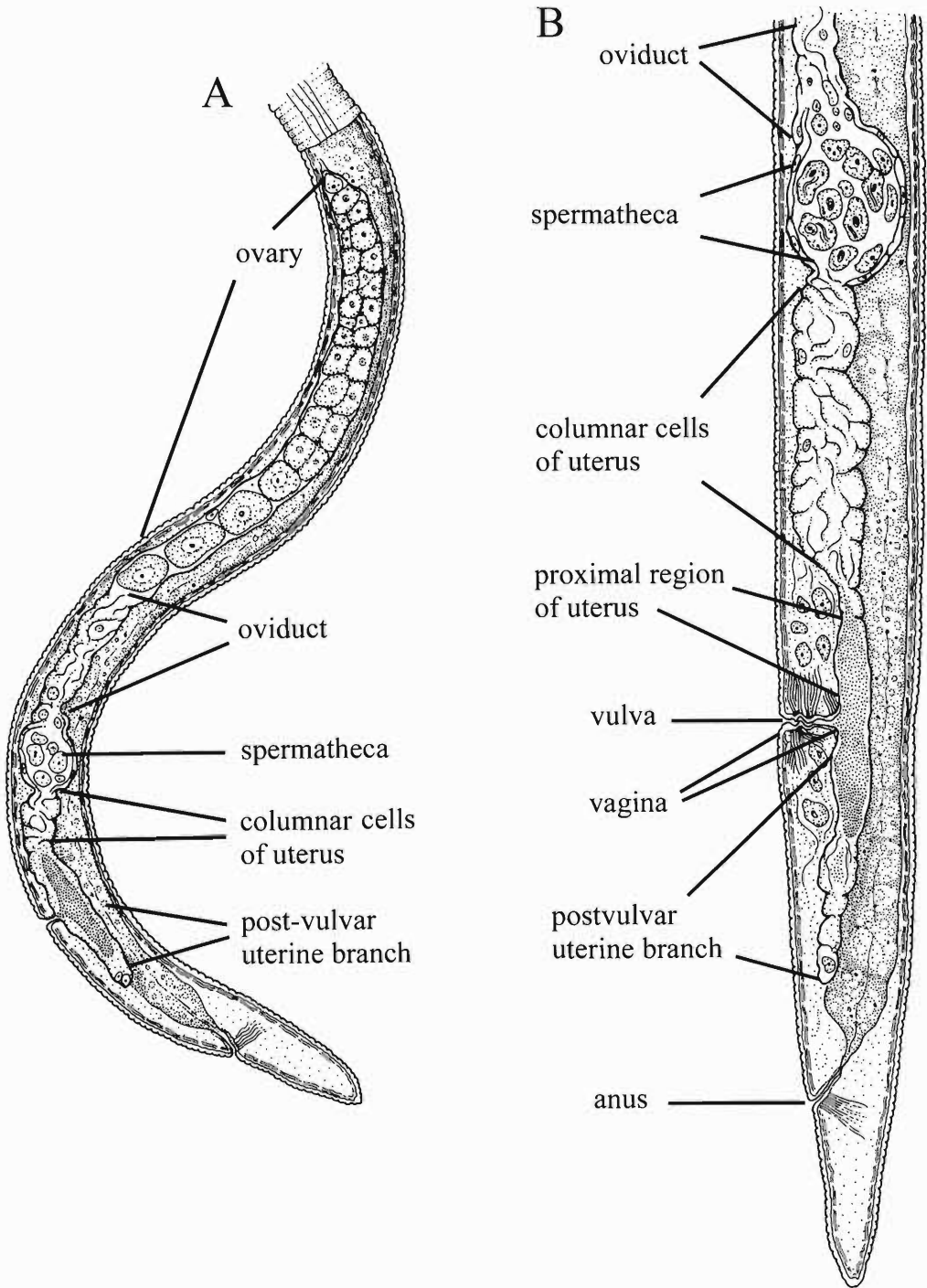
The oviduct (Fig. 11 on Foldout 4) consists of a series of irregularly shaped cells having plicated plasma membranes. Although adjacent cells are generally separated by many intercellular spaces, membrane junctions interconnect the cells and allow for the extensive opening of the oviduct that is required during passage of the enlarged oocytes. Muscle filaments are associated with most of the cells along the length of the oviduct (Fig. 9). Oviduct cells contain mitochondria and nuclei with irregularly shaped nuclear membranes lined with electron-dense chromatin. The cells occupy the ventral region of the body cavity and lie adjacent to the intestinal epithelium (Fig. 11). In the distal portion of the oviduct, the cells are more tightly packed and have centrally located membrane junctions (Fig. 12). In this region, the cells are not associated with muscle filaments. These closely arranged cells appear to function as a valve for the entry of oocytes into the spermatheca. Sperm were not observed on this side of the spermatheca.

## Spermatheca

The terminal cells of the oviduct are attached closely to spindle-shaped cells of the spheroid spermatheca (Fig. 11). Membranes of the cells of the spermatheca are joined together with prominent lateral membrane junctions. Spermatozoa in the center of spermatheca have prominent masses of chromatin that are surrounded by clusters of mitochondria and widely dispersed fibrillar bundles (Fig. 11). These structures are similar to the major sperm protein bodies that have been identified and described in other nematode species (Shepherd et al., 1973). The spermatozoa seem to be suspended in a moderately electron-dense fluid similar in appearance to the contents of the vas deferens of males. The posteriad boundary of the spermatheca joins a series of columnar cells of the uterus (Figs. 11, 13).

## Columnar cells of the uterus

Columnar cells leading posteriad from the spermatheca have plicated limiting membranes (Fig. 15 on Foldout 5) similar to those of cells in the oviduct (Fig. 8) but differing by the ab-



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Figure 1. Line drawings of the female reproductive system of *Pratylenchus penetrans*, illustrating the features of pseudomonodelphic reproductive development. (A) Anterior region of the gonad containing oogonial cells and oocytes in growth phase. (B) Posterior region of the reproductive system showing a postvulvar uterine branch. Posteriad to the oocytes are the oviduct, spermatheca, columnar cells of the uterus, and the vaginal-vulval regions.

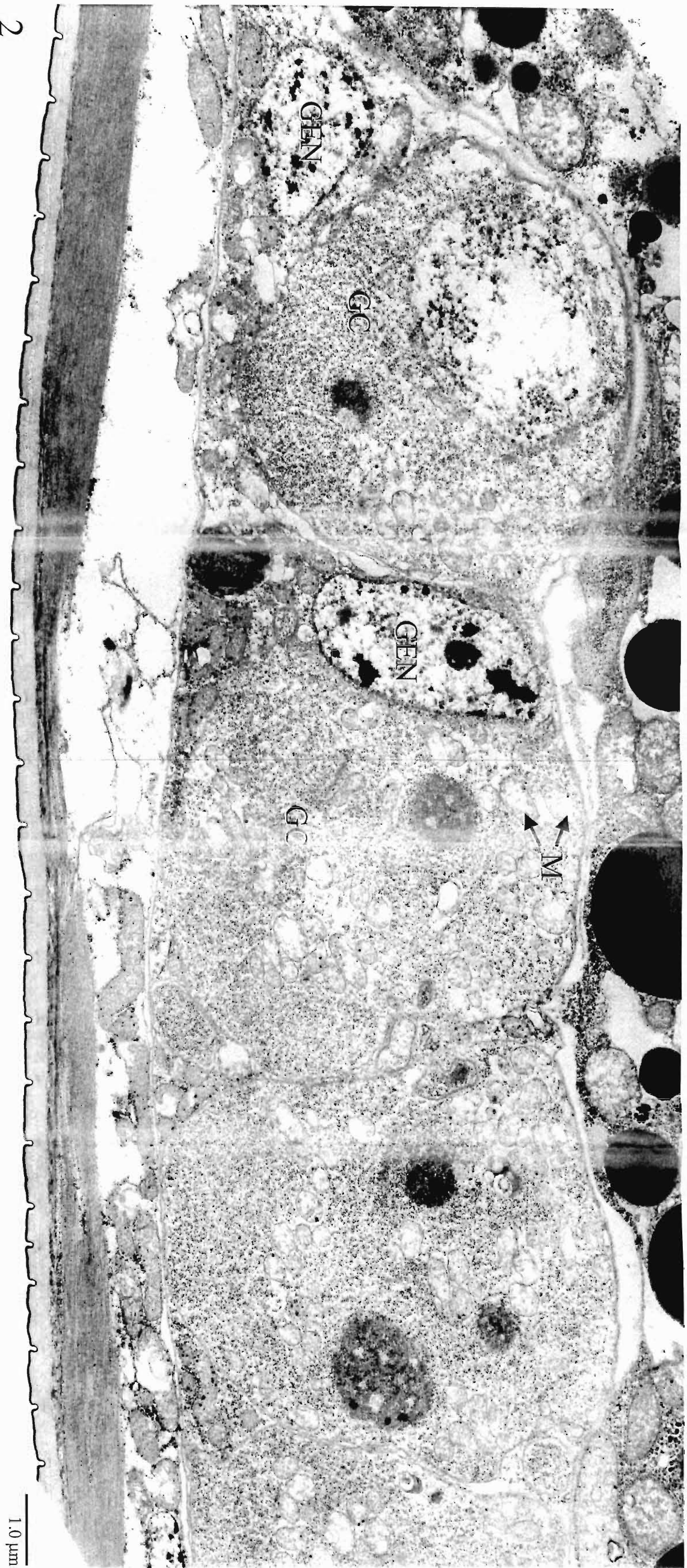


Figure 2. Distal region of the ovary of *P. penetrans* showing 3 enlarged distal cells. Germinal cells (GC) are precursors to oocyte development. Cells of the gonad epithelium lie adjacent to linearly arranged germ cells (i.e., oögonia and oocytes). GEN, gonad epithelium nucleus; M, mitochondria. (Note: In this and in later longitudinal figures that are illustrated with fold-outs (Figs. 2, 6, 8, 11, 15, 20), the proximal or left axis is toward the head of the nematode, whereas the distal or right axis is toward the tail. In the longitudinal sections that are illustrated in the single plates, the head to tail orientation is top to bottom.)





Figure 3. Longitudinal section through oocytes of *P. penetrans* in the region of their growth phase. Nucleoplasm with fragments of chromatin and electron-dense nucleolus (Nu). GEN, gonad epithelium nucleus; N, oocyte nucleus.



Figure 4. Longitudinal, submedian section of ovary of *P. penetrans* showing oocytes (O) during initial stages of meiosis. M, mitochondria; N, nucleus; SM, somatic muscles.

sence of muscle filaments within their cell boundaries. Membrane junctions between cells near the spermatheca define the region in which a lumen may form during oocyte passage (Fig.

13). However, a preformed lumen is not apparent. In this region, the spermatozoa may be displaced from the spermatheca as the oocyte moves through the central part of the uterus.

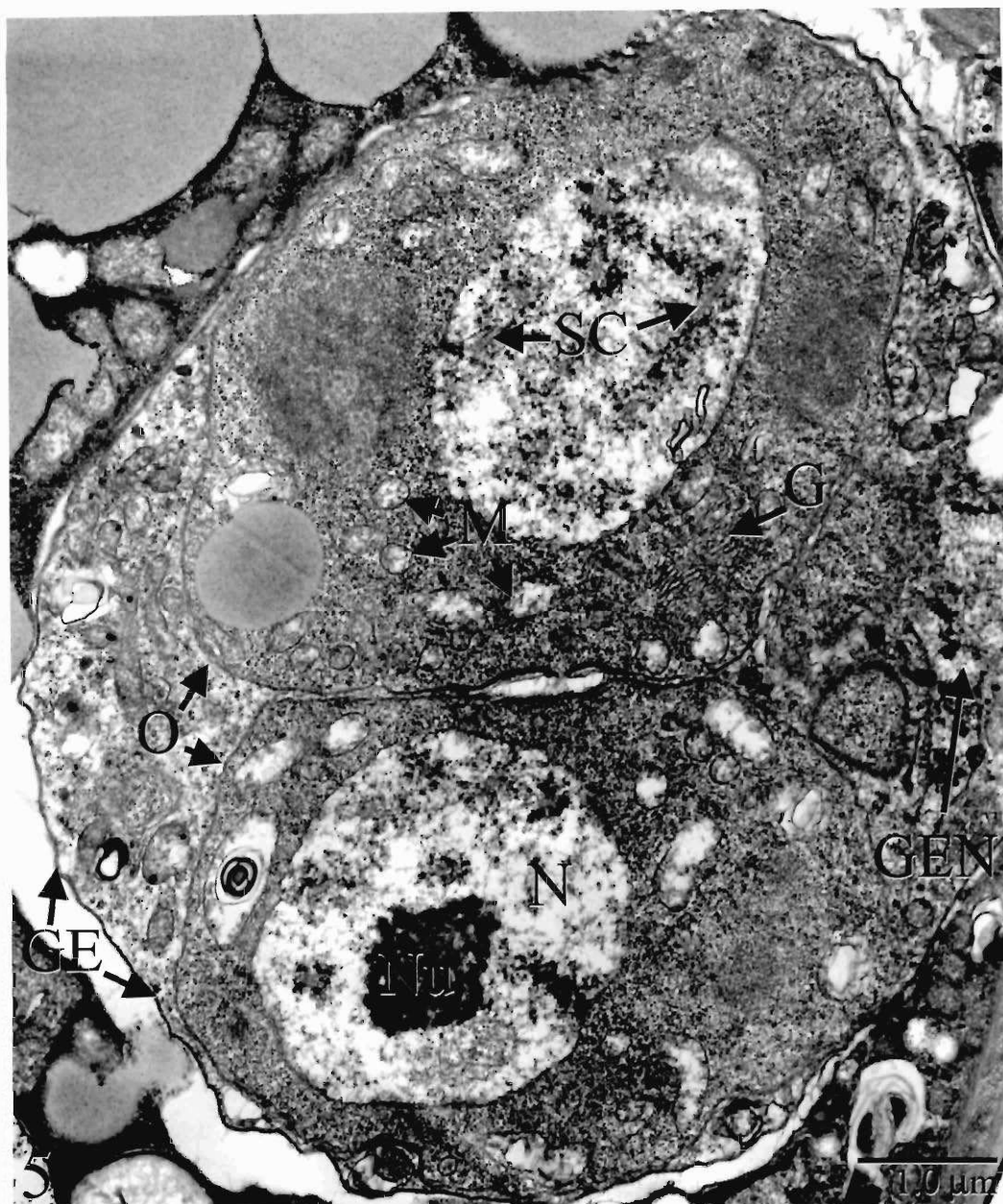


Figure 5. Transverse section of midregion of an ovary of *P. penetrans*, showing oocytes at pachytene stage of meiosis. Oocytes (O) are surrounded by gonad epithelial cells (GE), whose cytoplasm extends between the germinal cells. GEN, gonad epithelium nucleus; G, Golgi apparatus; M, mitochondria; Nu, nucleolus; N, nucleus; SC, synaptonemal complex.

When this occurs, the invagination of the plasma membrane of the columnar cell results in a spermatozoan that appears to have a double membrane (Fig. 13). Fertilization was not evident in the oocytes observed. Columnar cells of the

uterus have dense clusters of mitochondria and numerous polyribosomes throughout the cytoplasm (Figs. 13, 15). The distal columnar cells of the uterus contain relatively large clusters of electron-dense material (Fig. 15). The lumen,





Figure 6. Longitudinal section of the proximal region of the ovary of *P. penetrans*, illustrating growth stage of oocytes. CE, gonad epithelium; Nu, nucleolus; N, nucleus.





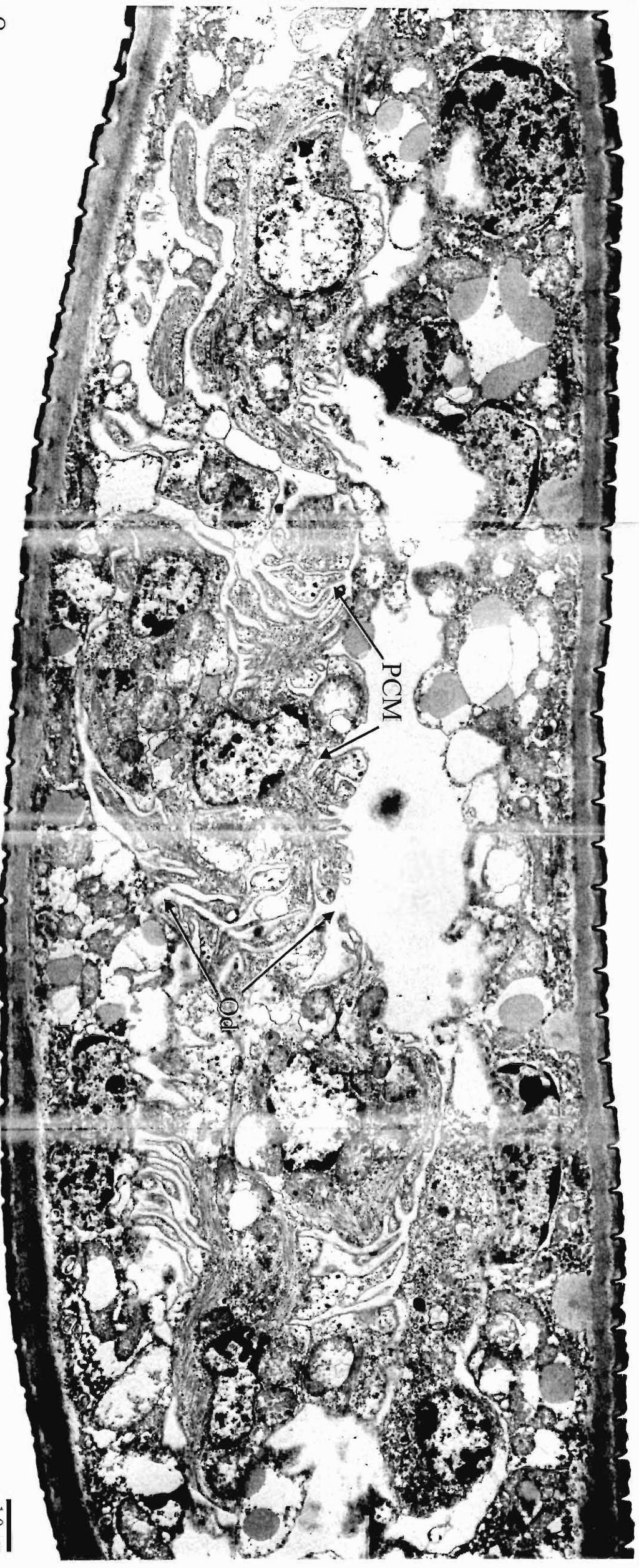
Figure 7. Longitudinal section of oocytes in proximal region of ovary of *P. penetrans*. An oocyte lies adjacent to the plicated cell membrane of the oviduct (Od).

which forms as the egg passes into the columella, merges with the central, fluid-filled channel of the uterus (Fig. 15). The main channel of the uterus continues posteriad as a flattened or collapsed region that extends across the ventral sector of the body, terminating in a postvulvar uterine branch (Figs. 17–19). The uterus opens ventrally through the cuticle-lined vagina and vulva (Fig. 16).

#### Egg passage

The traversing of an oocyte or egg through the spermatheca or between columnar cells compresses epithelial cells (Figs. 14, 20 on Foldout 6). In the absence of an egg within the uterus, the abundance of mitochondria and ribosomes and the occurrence of scattered secretory glob-

ules suggest that the columnar cells are metabolically active (Fig. 15). In the presence of an egg in the uterine channel, secretory granules occur intracellularly in compressed regions of uterine cells and extracellularly in the space between the surface of the egg and the limiting membrane of the columnar cells (Fig. 20). The accumulated secretory granules appear to contribute to the electron-dense deposits that form the egg shell. These deposits (Figs. 20, 21 on Foldout 6) accumulate on the vitelline layer, which is derived from the oolemma and has a unit membrane-like structure. Just below the vitelline layer is a chitinous layer followed by a lipid layer. The egg shell appears to be separated from the egg cytoplasm by a unit membrane. Tangential sections through the egg revealed



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Figure 8. Longitudinal section of oviduct of *P. penetrans*. Plicated cell membranes (PCM) form cisternae-like invaginations among the enlarged irregularly shaped cells along the oviduct (Od), which lacks a preformed lumen.

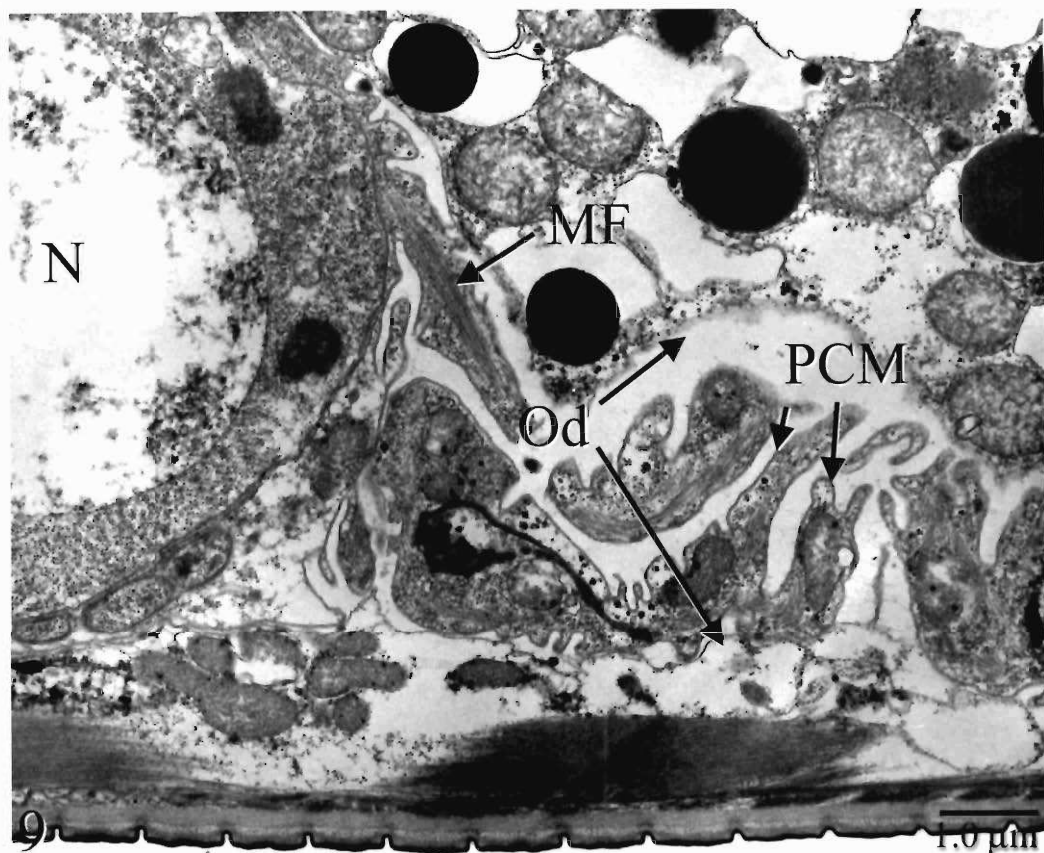


Figure 9. Longitudinal section of *P. penetrans* showing the junction between an oocyte and the oviduct (Od) supported by plicated cell wall membranes (PCM). Some cells with plicated membranes are associated with muscle filaments (MF). N, nucleus.

electron-transparent lipid bodies, numerous electron-dense protein granules (Fig. 20), and the sperm or egg nucleus, which contains prominent chromatin (Figs. 20, 22 on Foldout 6).

#### The vaginal-vulvar region

The wall of the vagina is continuous with the body cuticle (Fig. 16). Hemidesmosomes attach pairs of broad muscle bands to antieriad and posteriad portions of the vulva cuticle. These 4 fiber bands extending antieriad are believed to correspond to the anterior dilatores vulvae, whereas the posteriad muscle fibers are the posterior dilatores vulvae (Fig. 16). The muscle bands project ventrolaterally and connect with somatic muscles along the body cuticle (Fig. 17). Adjacent and internal to the vulva wall muscles is a broad band of sphincter muscles or the constrictor vaginae. Adjacent and dorsal to the constrictor

muscles are the anterior and posterior dilatores vaginae (Fig. 16). The cuticle of the vaginal wall is continuous with the ventral lining of the uterine channel.

#### Anal region

The body wall cuticle forms the lining of the anus and invaginates into the body cavity to form the lining of the rectum, which extends dorsoanteriad and subterminally into the tail region (Fig. 23). Proximally, the cuticular rectal channel is flat and broad (Fig. 25); distally it becomes elongate and oblong (Fig. 24). The noncuticularized region of the lumen is supported by rectal cells. The lumen may also be occluded by membrane evaginations of rectal cells joined laterally by membrane junctions. The H-shaped conformation of cells surrounding the rectum (Figs. 24, 25) coincides with the position



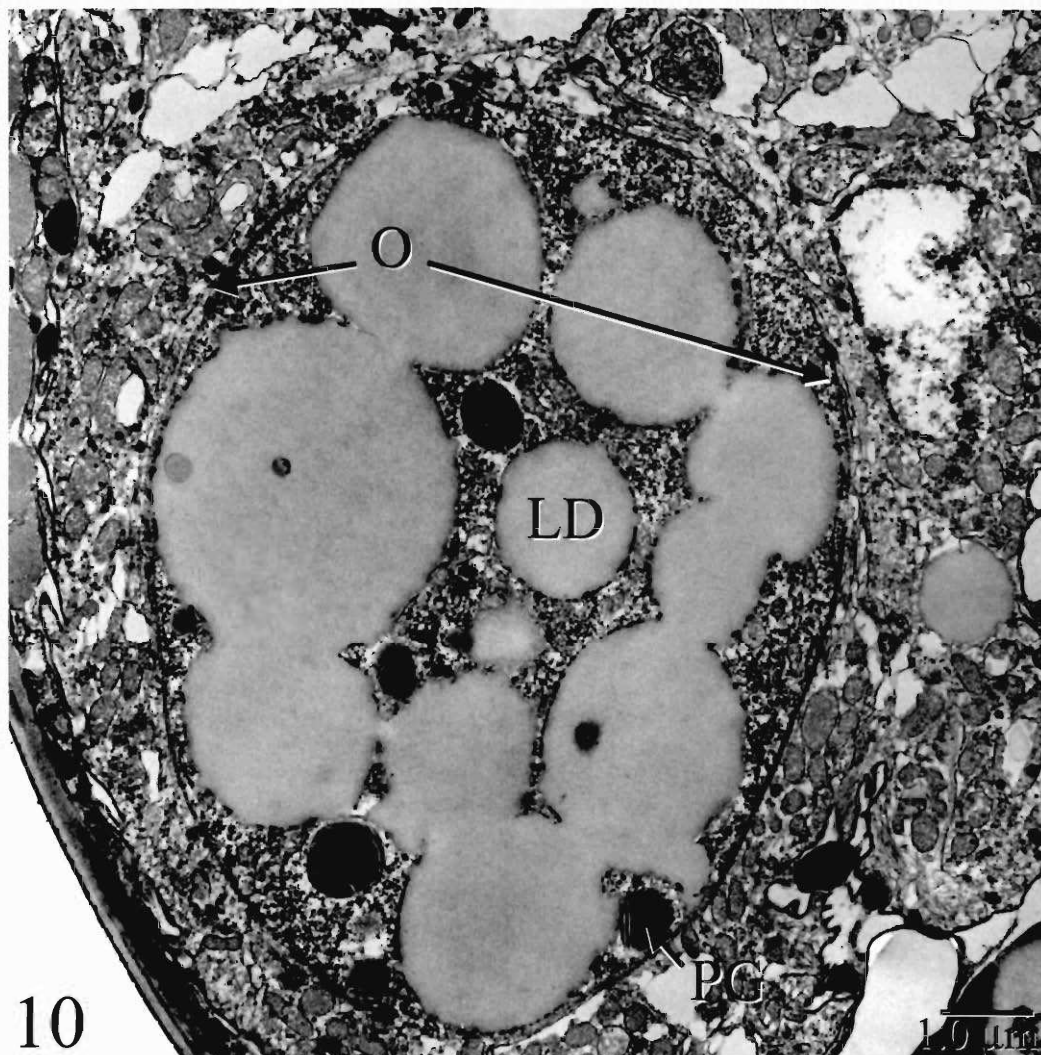


Figure 10. Transverse section of an oocyte (O) within the oviduct of *P. penetrans*. The oocyte is filled with large merging lipid droplets (LD) and a few protein granules (PG) that lie within the cytoplasmic matrix.

of the depressor ani muscles that connect the dorsal rectal cuticle to the dorsal lateral body cuticle via hemidesmosomes.

#### Discussion

In a study of postembryogenesis, Roman and Hirschmann (1969a) determined that several species of *Pratylenchus*, including *P. vulnus*, *P. coffeae*, *P. penetrans*, *P. brachyurus*, *P. zeae*, *P. neglectus*, and *P. crenatus*, have an amphidelphic pattern of gonad development. However, the monosexual species *P. scribneri* follows a mon-

odelphic pattern. In the amphidelphic species, 2 gonads develop until the fourth molt, then the posterior gonad deteriorates. The remaining gonad is prodelphic, similar to that of *P. penetrans*. The distal end of the telogonic gonad is occupied by an ovary with a short germinal zone and an elongated growth zone. The germinal zone contains oogonial cells that undergo rapid mitotic divisions. In the growth zone, the oocytes enlarge. The ovary is followed by a narrowly folded oviduct that is connected to the spermatheca by a 12-celled constriction (Roman and



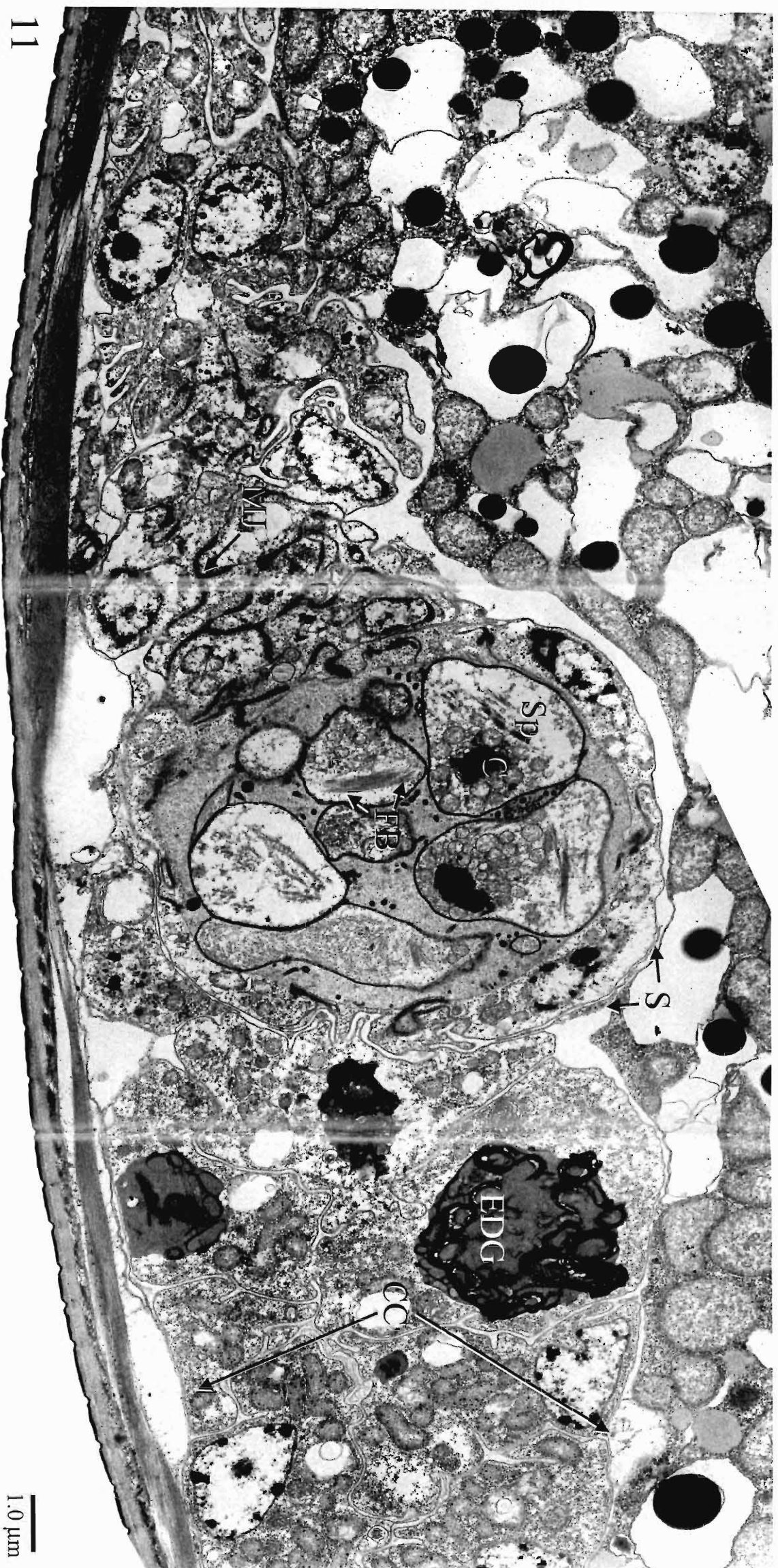


Figure 11. Longitudinal section of spermatheca of *R. penetrans*. The anterior boundary of the spermatheca (S) is surrounded by epithelial cells that are joined by membrane junctions (MJ). Spermatzoa (Sp) within the spermatheca contain electron-dense chromatin (C), mitochondria, and fibrillar bundles (FB). The posterior boundary of the spermatheca merges with enlarged columnar cells (CC) of the uterus. Columnar cells near the spermatheca contain enlarged electron-dense globules (EDG), numerous mitochondria, and ribosomes.

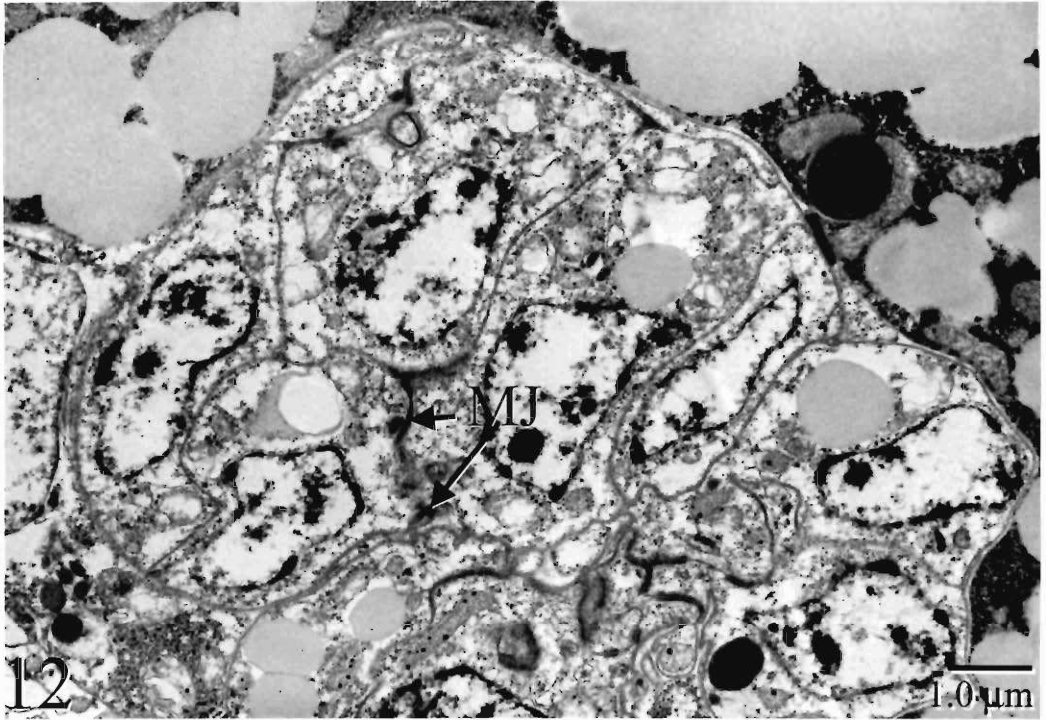


Figure 12. Transverse section through cells at the proximal region of the oviduct and anterior region of the spermatheca of *P. penetrans*. Membrane junctions (MJ) join adjacent cells so that a lumen is formed for the oocyte passage into the spermatheca.

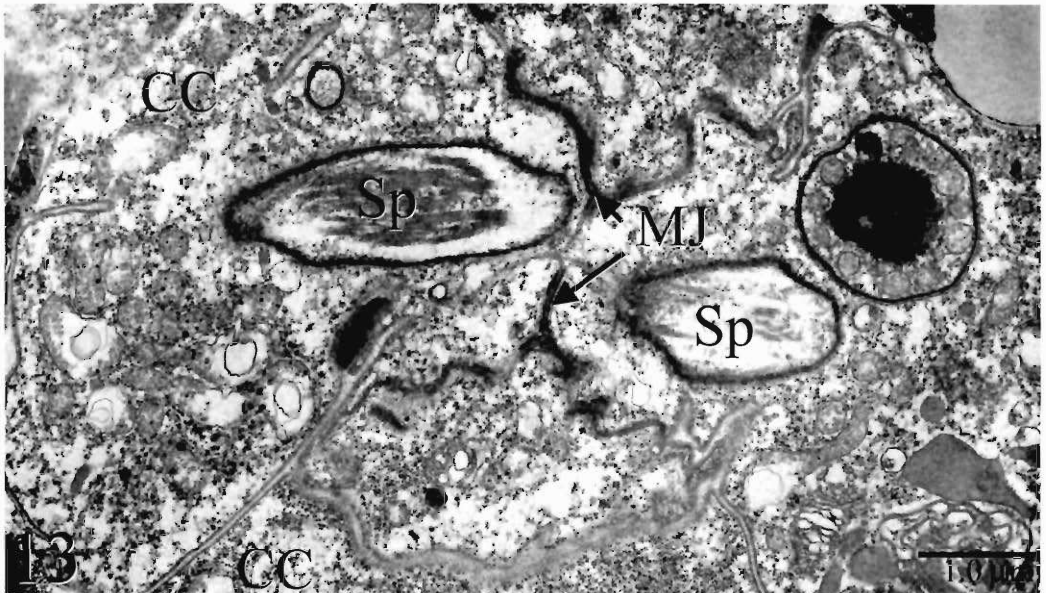


Figure 13. Transverse section near the posteriad region of the spermatheca of *P. penetrans*. Spermatozoa (Sp) appear to be outside the spermatheca and in the invaginated boundaries of the columnar cells (CC). Membrane junctions (MJ) result in adjoined cells that will form the lumen for egg passage.



Figure 14. Longitudinal section of an egg emerging from a spermatheca in a female *P. penetrans*. Spermatozoa (Sp) remaining in spermatheca (S) after oocyte passage appear to be oriented toward the emerged egg (E). Their electron-dense nuclei (N) and mitochondria adhere to the internal surface of the leading membrane of the spermatozoa. The membrane trailing the major body of the egg is intact and clearly separated from the spermatheca and its contents.



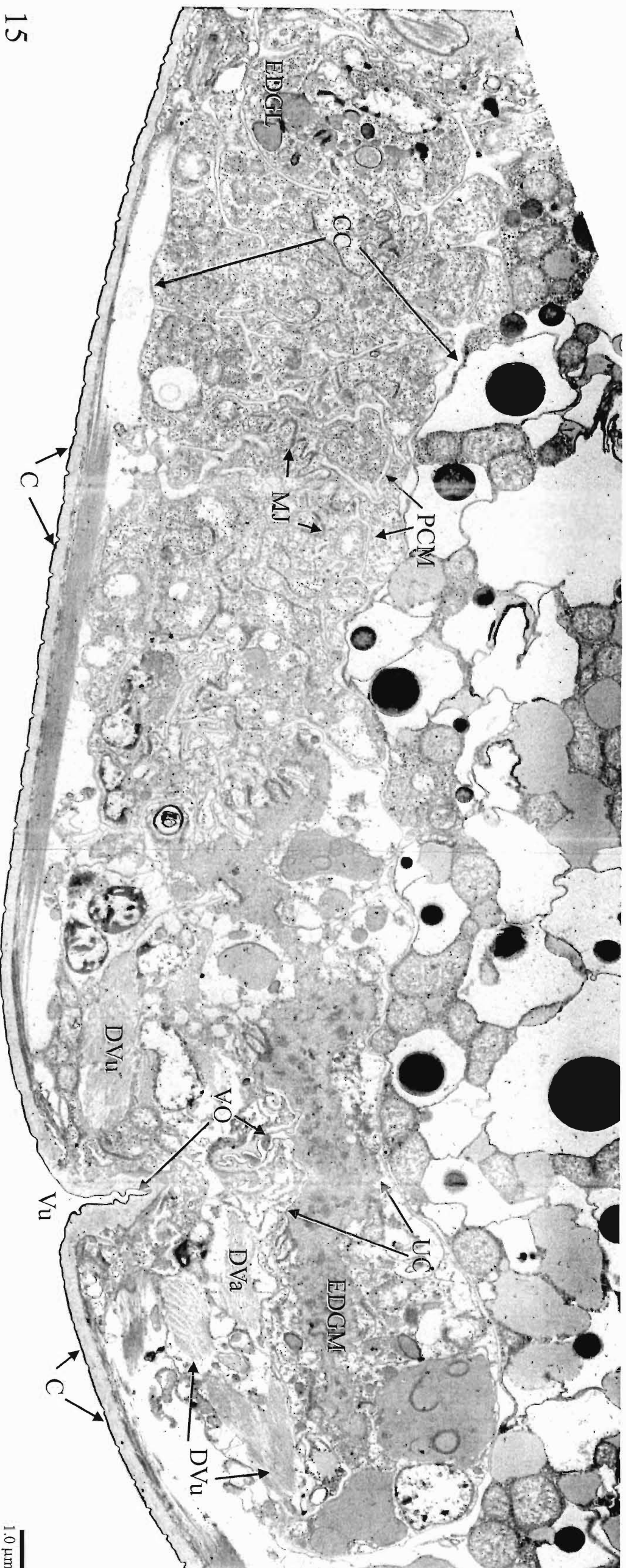


Figure 15. Submedian longitudinal section of columnar cells (CC) that delineate the lumen in the distal region of the uterus. The uterine channel (UC) posteriad from the columnar cells is filled with electron-dense granular material (EDGM) that extends from beyond the vagina into the postvulvar uterine region of the reproductive system. This submedian section illustrates the continuity of the lining of the vaginal lumen (VO) with the body wall cuticle (C), but not with the lumen of the uterus. Tangential sections show muscle fibers that belong to the dilator vaginae (DVa) and dilator vulvae (DVu), which play a major role in egg deposition. EDGL, electron-dense globules; MJ, membrane junctions; PCM, plicated cell membranes; Vu, vulva.



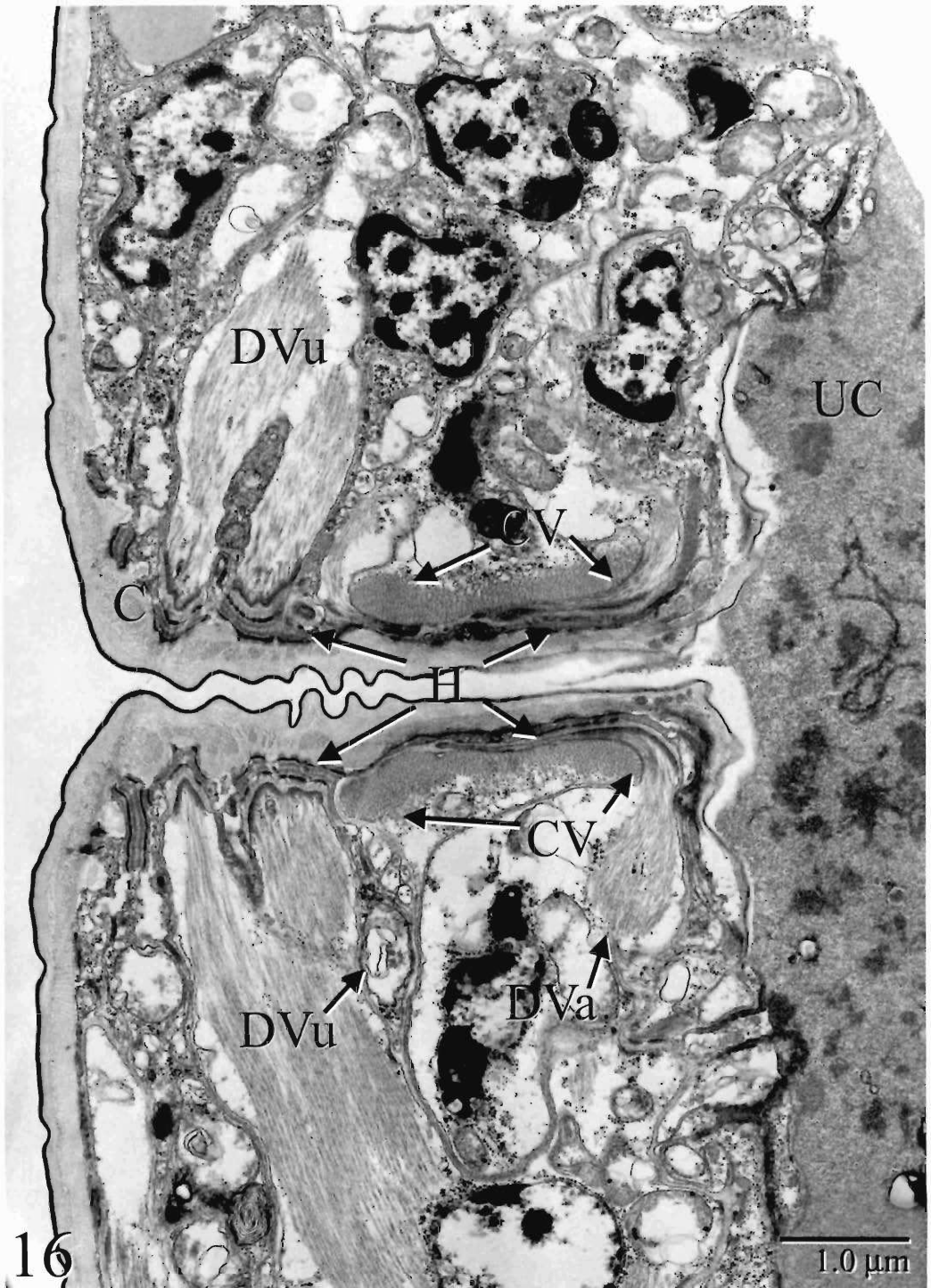


Figure 16. Longitudinal section of the uterus and vagina of *P. penetrans*. The cuticular lining of the vagina is continuous with the body wall cuticle (C) and extends internally to join the uterine channel (UC). Hemidesmosomes (H) attach the dilatores vaginae (DVa) and dilatores vulvae (DVu) muscles to the cuticle lining the vulva and vagina. Constrictores vaginae (CV) or sphincters surround and attach to the cuticle that forms the inner region of the vagina.

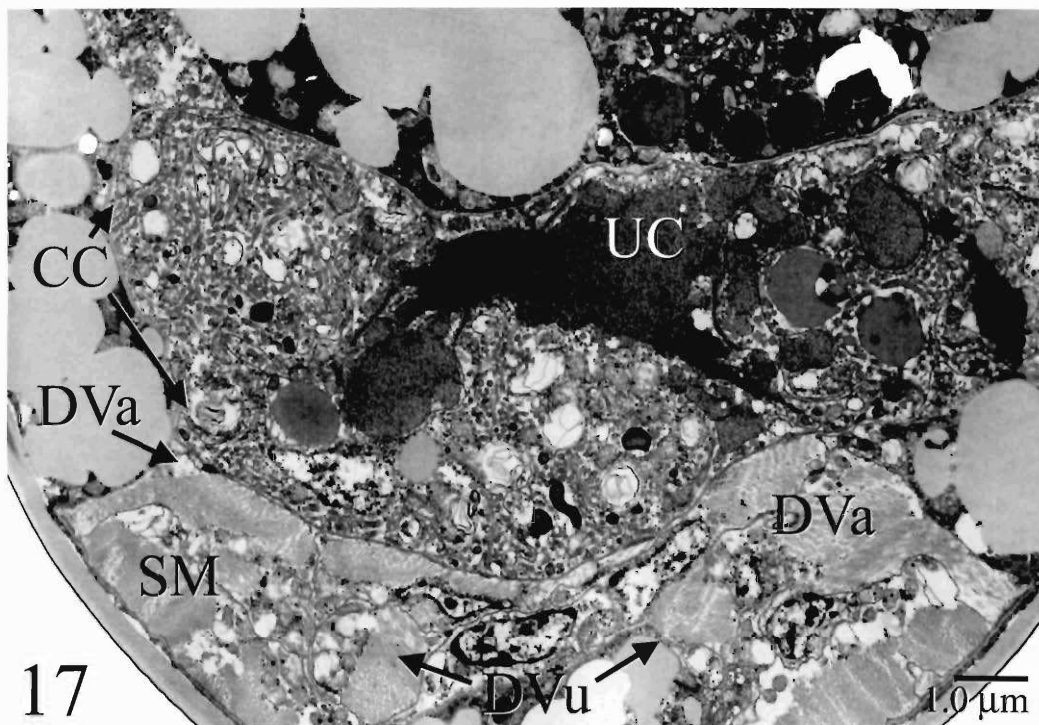


Figure 17. Transverse section through columnar cells (CC) of the uterus surrounding the proximal end of the uterine channel (UC) of *P. penetrans*. Muscle elements adjacent to the columnar cells are extensions of dilatores vaginae (DVa) or dilatores vulvae (DVu) muscles. Other extensions of these muscles (DVa and DVu) contact the laterosubventral somatic muscles. SM, somatic muscles.

Hirschmann, 1969b). The spermatheca, which is composed of about 10 epithelial cells, is followed by the uterus that consists of 2 portions. The distal portion is composed of 12 large gland cells arranged in 4 rows of 4 cells each (tricolumella) that could have a role in egg shell deposition. The proximal portion is a short tube lined with a flat epithelium. This portion enters the vagina, which is lined with cuticle and supported by muscles and opens through the vulva. Specialized muscles dilate the vulva during oviposition (Hirschmann, 1971).

In the present study, the ultrastructural observations were of adult specimens of *P. penetrans*. The morphology of the reproductive system that we observed is similar to the modified amphidelphic mode of development described in previous studies. Briefly, in *Pratylenchus crenatus* and *P. penetrans* (Dickerson, 1962) and in a diverse group of *Pratylenchus* species (Roman and Hirschmann, 1969a), the reproductive system is comprised of a functional anterior ovary and a posterior branch of the ovary reduced to a post-

vulvar uterine branch. The mitotic divisions occur in the blunt anterior terminus of the developing ovary (Coomans, 1962; Dickerson, 1962; Hirschmann, 1962; Yuen, 1964; Roman and Hirschmann, 1969a). Mitotic divisions were not observed in distal cells of the ovary. These events may occur rather quickly and may not have been captured at our fixation times. No distinction could be made between oogonial and oocyte cells in the anterior region of the ovary in several mature female specimens. However, lipid accumulations were observed among oocytes in the oviduct of an actively reproducing female. In addition, our observations suggest that extracellular lipid or protein granules could nourish the oocyte.

Future work should involve labeling experiments to show the movement of secretory granules from ovarian epithelial cells across the limiting membranes of the oocyte. If this movement occurs, it could explain the accumulation of lipids and proteins that are associated with oocyte enlargement. Changes also appear to occur in

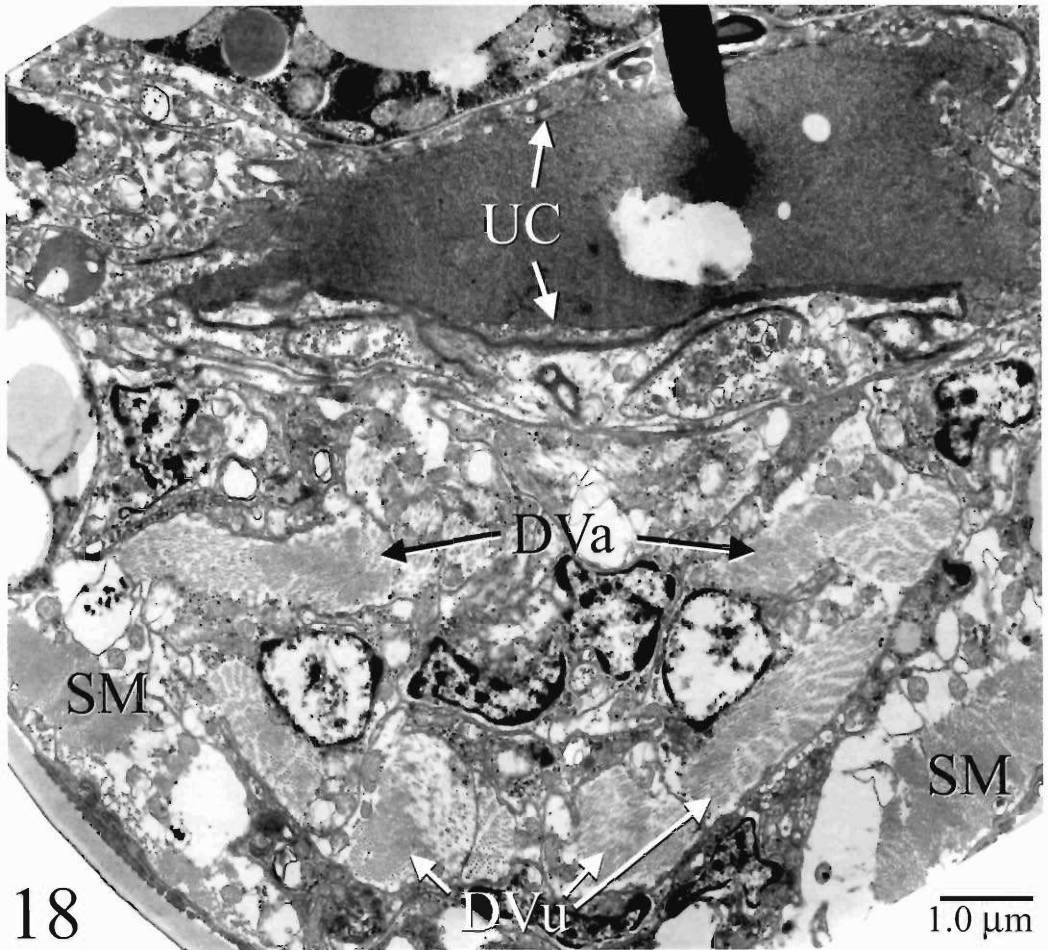


Figure 18. Transverse section of the uterine channel (UC) and muscles associated with the dilation of the vagina and vulva during egg deposition by *P. penetrans*. DVa, dilatores vaginae; DVu, dilatores vulvae; SM, somatic muscles.

the morphology and porosity of the oocyte surface as it passes through the spermatheca, becomes fertilized by sperm, and begins to receive egg shell depositions from columnar cells prior to egg deposition. The electron-dense globules observed in some cells of the distal region of the columnar cells are unusual and are dissimilar to secretory granules observed in the cells forming the oviduct and proximal regions of the uterus.

In our study, nuclear divisions were not observed in the distal region of the ovary of the mature females. This observation is consistent with the observations of Roman and Hirschmann (1969a), who found that oögonial divisions occur in the germinal zone of the ovary of

fourth-stage juveniles and probably in young females, but not in mature, egg-laying females. Similarly, oögonial divisions were observed in populations of the soybean cyst nematode, *Heterodera glycines*, before and during the fourth molt. This species has a normal meiotic cycle and reproduces by cross-fertilization (Triantaphyllou and Hirschmann, 1962).

The presence of synaptonemal complexes in many of the ovary cells proximal to the anterior end indicate that many of the oocytes are in the pachytene stage of prophase I. The presence of the tripartite synaptonemal complex is consistent with observations of nuclei in the testes of *P. penetrans*. This tripartite pattern differs from that of most species of *Meloidogyne*, which have



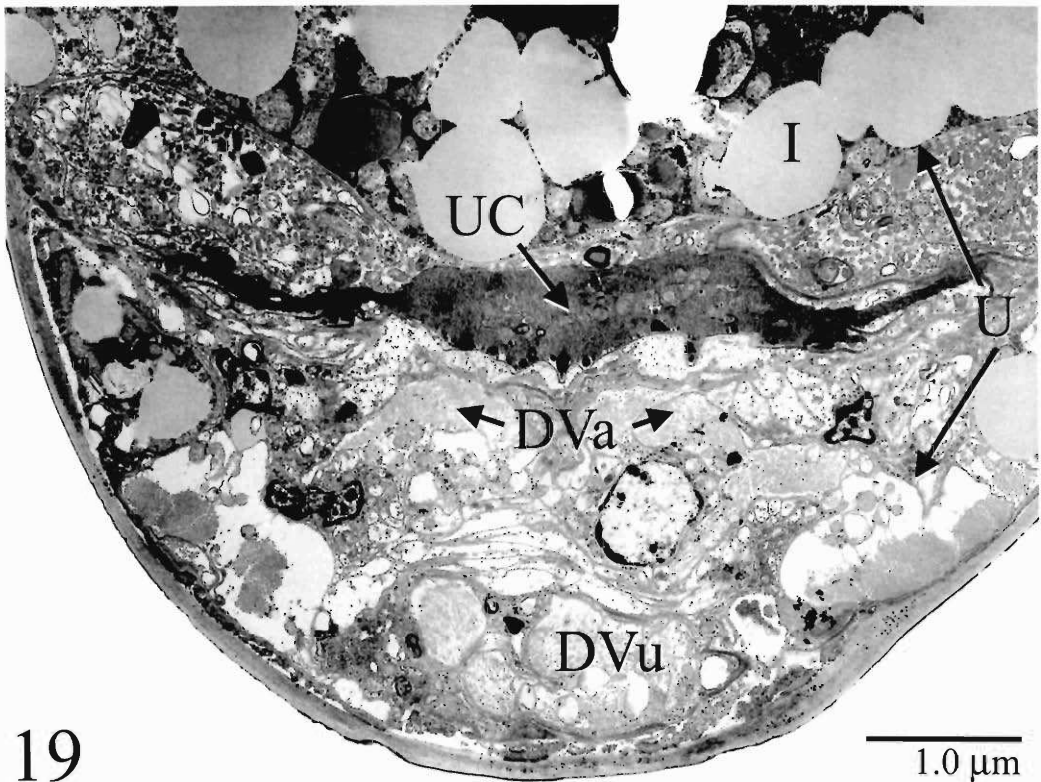


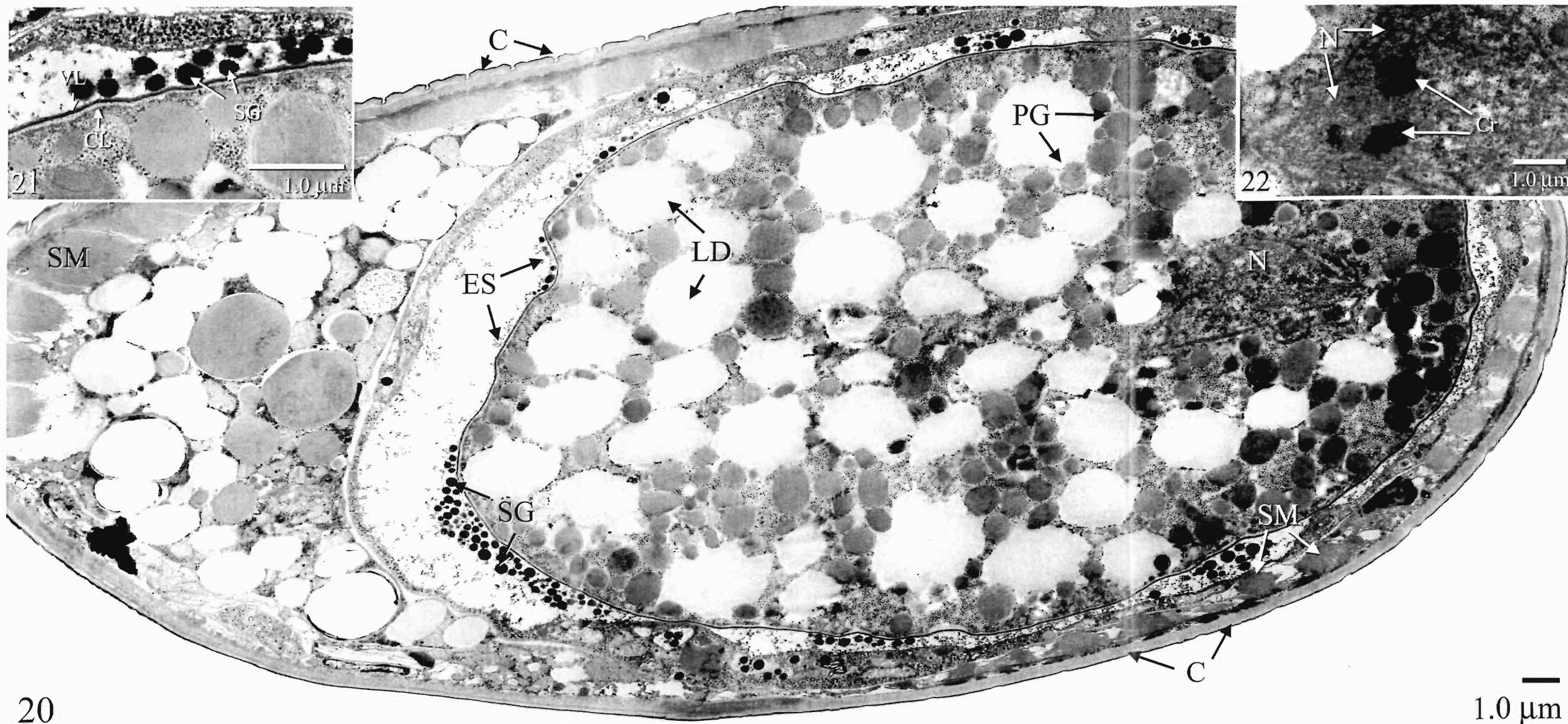
Figure 19. Transverse section through the uterine channel (UC) of *P. penetrans* showing the broad opening for egg passage. The bands of muscles adjacent to the uterine channel are the dilatores vaginae (DVa). The bands of muscles midventral and close to the body wall cuticle constitute the vulval wall muscles, dilatores vulvae (DVu). I, intestine; U, uterus.

a bipartite pattern consisting of 2 lateral elements but lacking striated central elements (Westergaard and von Wettstein, 1972; Goldstein and Triantaphyllou, 1995). Whether or not the tripartite pattern of the synaptonemal complex occurs in most species of *Pratylenchus* is not yet determined. Observations of *Caenorhabditis elegans* show that developing oocytes are arranged in single file along the proximal arm of the ovary, the site of gametogenesis in a hermaphrodite. Oocytes are arrested at diakinesis in meiotic prophase I. After the oocyte is fertilized, the zygote moves through the spermatheca to the uterus, where meiosis is completed (Kimble and Ward, 1988).

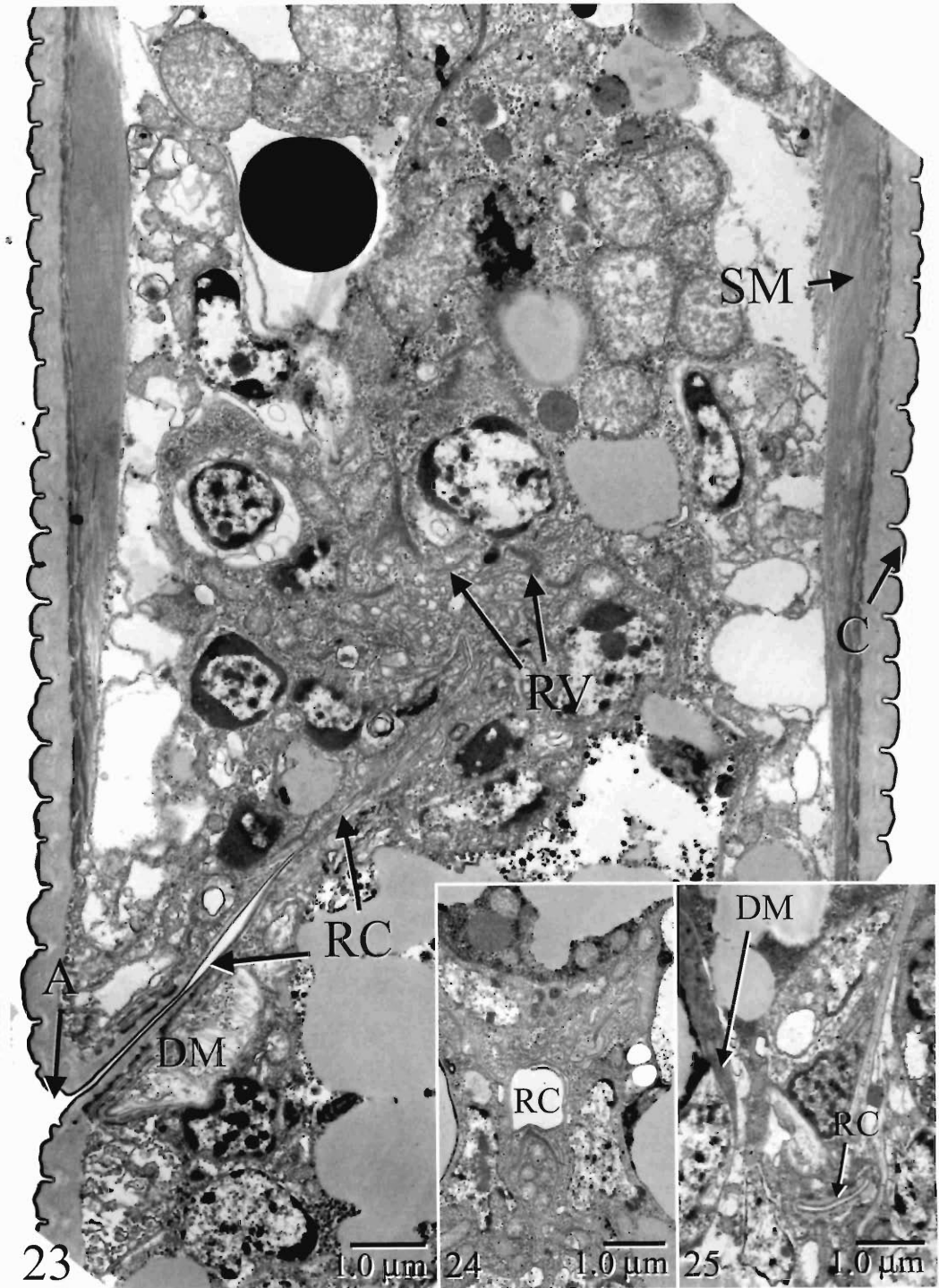
In *Xiphinema theresiae*, the ovary has 2 types of cells: the ovarian epithelial cells and the germ cells (Van De Velde and Coomans, 1988). The ovarian epithelial cells form a thin layer around the germ cells and have nuclei between some of the germ cells. At some sites, processes of ovar-

ian epithelial cells extend inward to form a central cytoplasmic mass, which has cytoplasmic contact with the germ cells. These cells develop 2 membrane-derived features, the villi and the small coated bulges, which are thought to play a role in transport. However, *X. theresiae* does not have a typical rachis, a large, clearly delineated structure, around which oogonia are arranged and make cytoplasmic contact.

Bird and Bird (1991) described a typical rachis for the telogonic and didelphic reproductive system of the female root-knot nematode, *Meloidogyne javanica*. The oogonia are radially arranged around a central anucleate rachis to which oogonia are attached by cytoplasmic bridges. In *C. elegans*, which is monodelphic, mitotic germ cells occupy the distal end of the ovary, and meiotic cells occupy the remaining portion of the gonad (Kimble and White, 1981). A typical rachis was not observed in the female reproductive system of *P. penetrans*.



Figures 20–22. Section of an egg of *Pratylenchus penetrans*. 20. Tangential section through an egg within the postvulvar uterine branch. This section is from the same specimen shown in Figure 10, which shows an oocyte in the oviduct. The oocyte cytoplasm is filled primarily with lipid bodies. In contrast, the cytoplasm of the egg contains numerous irregularly shaped electron-translucent lipid droplets (LD) and numerous electron-dense protein granules (PG) that generally occur around the lipid droplets. The nucleus (N) is located near one end of the egg. The egg shell (ES) has a well-defined electron-dense outer vitelline layer and an electron-translucent inner chitinous layer. Electron-dense secretory granules (SG) accumulate on the surface of the egg. Similar granules occur singly or in clusters within the columnar cells of the uterus or supporting cells of the uterine channel. The intercellular electron-dense granules originating in these cells appear to be secreted and deposited on the egg shell. C, cuticle; SM, somatic muscle. 21. Enlargement of a portion of the egg shell shown in Figure 20. Secretory granules (SG) near the surface of the egg shell appear to contribute to the electron-dense outer vitelline layer (VL), which is easily distinguished from the electron-translucent inner chitinous layer (CL). 22. Enlargement of nucleus (N) of egg shown in Figure 20. Chromatin (Cr) occurs within the nucleoplasm.



Figures 23–25. Section of the rectal region of *Pratylenchus penetrans*. 23. Longitudinal section of the rectal region of an adult female. The complex of membrane junctions denotes part of the rectal valve (RV). The rectal channel (RC) extends posteriad and is supported by the cell membranes and cuticle. The depressor ani muscles (DM) are located on the dorsal surface of the rectal cuticle near the anal (A) opening. SM, somatic muscles; C, cuticle. 24. Transverse section of the rectal channel (RC) supported by rectal cells. 25. Transverse section of the rectal channel (RC) near the attachment of the depressor ani muscles (DM) to the cuticle lining of the channel. The depressor ani muscles have a dorsosublateral orientation.



The ovary of *P. penetrans* has cells that appear as single or double rows of germ cells enclosed by epithelial cells that are characterized by irregularly shaped nuclei. These nuclei have electron-dense chromatin that tends to accumulate along the inner surface of the nuclear membrane. Cytoplasmic contact between germ cells and epithelial cells appears to be minimal and is not similar to that described for other species (Hirschmann, 1971). The distinctive morphology of epithelial cells of *P. penetrans* ovaries was also noted in the cluster of cells antieriad to the spermatheca. These epithelial cells, in conjunction with oviduct cell wall, may affect movement of oocytes from the oviduct into the spermatheca.

The plicated membranes of cells lining the oviduct and their capacity to expand and accommodate the moving oocyte were previously illustrated for *Rotylenchus goodeyi* (Coomans, 1962) and the Hoplolaiminae (Yuen, 1964). This process may also operate in the spermatheca and columnar cells. However, a fundamental difference occurs in their cellular contents and functions. In *P. penetrans*, the presence of muscle filaments, which line the oviduct, suggests that they have an active role during oocyte passage toward the spermatheca. The cluster of cells, which have centralized membrane junctions at the anterior region of the spermatheca and are described as a 12-celled constriction in *Pratylenchus* spp. (Roman and Hirschmann, 1969b), may function as a valve, which opens or closes to regulate oocyte passage into the spermatheca. The female reproductive system of *Xiphinema meridianum* has an ovarian sac that is muscular and an outer membrane that is highly plicated. The proximal part of the oviduct is narrow and tube-like, but widens into the pars dilatata oviductus. The oviduct of *X. meridianum* lacks a preformed lumen except for the pars dilatata oviductus, where the lumen is narrow. The ultrastructure of the female gonoduct of *X. theresiae* is similar to that described for *X. meridianum* (Van De Velde et al., 1990a, b). In *P. penetrans*, the ultrastructure of the lumen of the oviduct and that of the columnar cells in the central region is similar to the plicated cell membranes described for *Xiphinema*, which also lacks a preformed oviduct lumen.

Ward and Carrel (1979) described oocyte migration in the hermaphroditic species *C. elegans*. In this species, migration is accompanied by

sporadic contractions of the oviduct walls and the oocyte cytoplasm. As contractions of the oviduct wall increase, the oocyte moves through the spermathecal constriction and into the spermatheca. A similar mechanism may propel oocytes through the muscular oviduct of *P. penetrans*.

The spermatheca of *P. penetrans* is defined by the adjoining columella cells. Columella cells are joined by a junctional complex to form a continuous lumen between the spermatheca and the central uterus. The ultrastructure of the columella cells of the uterus is distinctly different from the cells forming the oviduct. In the uterus, the columella cells have more ribosomes, mitochondria, secretory granules, and membrane junctions than the cells adjoining the oviduct. In the female gonad of *Rotylenchus goodeyi*, the uterus has two regions: the quadricolumella and a thin-walled, muscular region that lies between the quadricolumella and the vagina (Coomans, 1962). This muscular region was not observed in *P. penetrans*. However, the muscle bands that were found near the vagina and vulva appear to have a major role in the movement of the oocyte or egg through the genital tract as well as in dilation of the vagina and vulva during egg deposition.

In a study of about 50 females of *R. goodeyi*, Coomans (1962) determined that the quadricolumella is a glandular region in the uterus and probably secretes the egg shell. The glandular region was particularly large and granular when a well-developed egg was found in the oviduct. As the egg passed into the uterus, the glandular cells appeared to empty and a thin layer formed around the egg shell. In *P. penetrans*, the uterus with eggs has electron-dense secretory granules in the columella cells, and cells of the uterine wall are appressed and flattened by passage of an egg. At this time, the secretory granules are found between the uterine wall and the limiting membrane of the egg.

We concur that the columella cells serve a functional role in providing secretions that contribute to formation of the egg shell, as proposed by Coomans (1962) for *R. goodeyi* and by investigators of other nematode species (Coomans, 1965; Blevé-Zacheo et al., 1976; McClure and Bird, 1976; Bird and Bird, 1991). This hypothesis is further supported by ultrastructural examinations of cross sections of egg shells of *P. penetrans* (Hilgert, 1976). Our study illustrates

sites where secretory granules appear to merge with the electron-dense outer layer of the egg shell.

Fertilization of the oocyte occurs between the oviduct and the uterus, regardless of the presence or absence of a spermatheca (Bird and Bird, 1991). In a light microscope study, Hung and Jenkins (1969) observed oogonial divisions at the apical portions of the gonads of young females of *P. penetrans* and *P. zaei*. In *P. penetrans*, only 1 sperm appears to enter each oocyte as it passes through the spermatheca. After sperm penetration, the oocyte nucleus moves centrally and undergoes maturation divisions. After the initial reduction division and the second division of meiosis, the egg pronucleus is formed, which in turn fuses with a sperm nucleus to form the zygote shortly before or after egg deposition. In *P. penetrans*, the chromosome number is  $2n = 10$ , whereas in *P. zaei*, which reproduces by mitotic parthenogenesis,  $2n = 26$ . In the present ultrastructural study of *P. penetrans*, the stage at which fertilization occurs could not be determined, but sperm was observed in the developing eggs inside the uterus.

In *Ascaris*, Foor (1970) showed that when male sperm and oocyte membranes establish contact, the membranes appear to fuse. In other cases, considerable interdigitation occurs between the opposing gamete surfaces. Subsequently, the sperm progresses to a position deep within the oocyte cytoplasm. In some cases, fusion appears to take place between the oolemma and the lateral margins of the sperm. After fusion of the gamete membranes, the underlying interdigitating membranes disappear and the contents of the spermatozoan are within the oocyte (Foor, 1970).

In *P. penetrans*, the ultrastructure of initial stages of gamete fusion was not examined. Hung and Jenkins (1969) used light microscopy to show that the oocyte nucleus of *P. penetrans* undergoes 2 divisions after sperm penetration. Roman and Triantaphyllou (1969) studied the maturation of oocytes and fertilization in *P. penetrans*, *P. vulnus*, and *P. coffeae*. In these species, oocytes in the spermatheca contain a small number of bivalent chromosomes at prometaphase I. One spermatozoan enters each oocyte, which then rapidly completes the first division. At telophase I, the chromosomes that form the first polar body nucleus are discrete and can be used to determine the haploid chromosome num-

ber. A second maturation division follows rapidly, and the sperm pronucleus is formed and then fuses with the egg pronucleus to form the zygote nucleus. Fusion of the pronuclei was observed in nondeposited eggs of *P. penetrans* and in eggs laid by *P. coffeae*. The primary oocyte of the dog heartworm, *Dirofilaria immitis*, completes meiosis only after fertilization by a male gamete in the seminal vesicle (Delves et al., 1986). After meiosis I and II are completed in the oocyte and the 2 polar bodies are extruded, the haploid chromosome complement of the female unites with that of the male and re-establishes the diploid chromosome number in the zygote.

Oogenesis and the mode of reproduction were also studied in populations of the soybean cyst nematode, *H. glycines*. Triantaphyllou and Hirschmann (1962) determined that oogonial divisions occur before and during the fourth molt. Maturation of oocytes in inseminated females consists of 2 meiotic divisions and the formation of 2 polar nuclei. Nine bivalents are present at metaphase I in all populations. Sperm enters the oocytes at late prophase or early metaphase I. After the second maturation division, sperm and egg pronuclei fuse to form the zygote nucleus.

The vulval walls of *P. penetrans* are attached to 2 sets of dilatores vulvae. Four bands on each side of the vulval wall are directed antieriad and posteriad and insert ventrolaterally on the body wall. This orientation of muscles coincides with light microscopic observations of *R. goodeyi* (Coomans, 1962). The dorsally and ventrally located dilatores vaginae have been diagrammed for *P. penetrans* (Kisiel et al., 1972; Hilgert, 1976; Mai et al., 1977). Although the vulval muscles were not clearly defined in the latter studies, they did appear as prominent muscle bands in our study.

In the hermaphrodite *C. elegans* stained with phalloidin, a photomicrograph clearly showed 4 of 8 vulval muscle cells that were inserted near the vulval opening and at the lateral epidermis (Waterston, 1988; Bird and Bird, 1991). Our observations of *P. penetrans* tend to support the concept that the dilatores vulvae play a major role in egg deposition.

In conclusion, the ultrastructure of the reproductive system of *P. penetrans* increases our understanding of the anatomical, physiological, and phylogenetic relations among a vast array of nematodes, including many plant parasitic nem-

atodes. In the future, comparative studies should be conducted on reproductive anatomy and physiology of sedentary endoparasitic species such as *Meloidogyne*, the cyst nematode species, and the migratory and free-living forms, such as *C. elegans*. These observations may provide clues for modifying or disrupting nematode reproduction and could lead to new methods of control for economically destructive species.

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## 1999–2000 MEETING SCHEDULE OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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| 13 October 1999  | Animal Parasitology Laboratories, Beltsville Agricultural Research Center, USDA, Beltsville, MD, 7:30 pm (Contact person: Eric Hoberg, 301-504-8588)                                      |
| 17 November 1999 | Anniversary Dinner—meeting location TBA   |
| 19 January 2000  | Smithsonian Institution, National Museum of Natural History, Washington, DC, 7:30 pm (Contact person: Bill Moser, 202-357-2473)   |
| 12 March 2000    | Johns Hopkins Montgomery County Center (Provisional), Rockville, MD, 7:30 pm (Contact person: Tom Simpson (JHU), 410-366-8814, or Louis Miller (NIH), 301-496-2183)                       |
| 10 May 2000      | Joint Meeting with the New Jersey Society for Parasitology, at the New Bolton Center, University of Pennsylvania, Kennett Square, PA, 2:00 pm (Contact person: Jay Farrell, 215-898-8561) |